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(54) **Antithrombotic compositions containing docosahexaenoic acid**

(57) A pharmaceutical composition or foodstuff contains docosahexanenoic acid or a salt, ester or amide thereof, to prevent or treat thrombosis. The compounds are obtained by extraction, concentration and isolation from aquatic fishes, crustacea, molluscs, mammals, algae or plankton. An antioxidant is preferably added to the composition, e.g. tocopherol or erythorbic acid.

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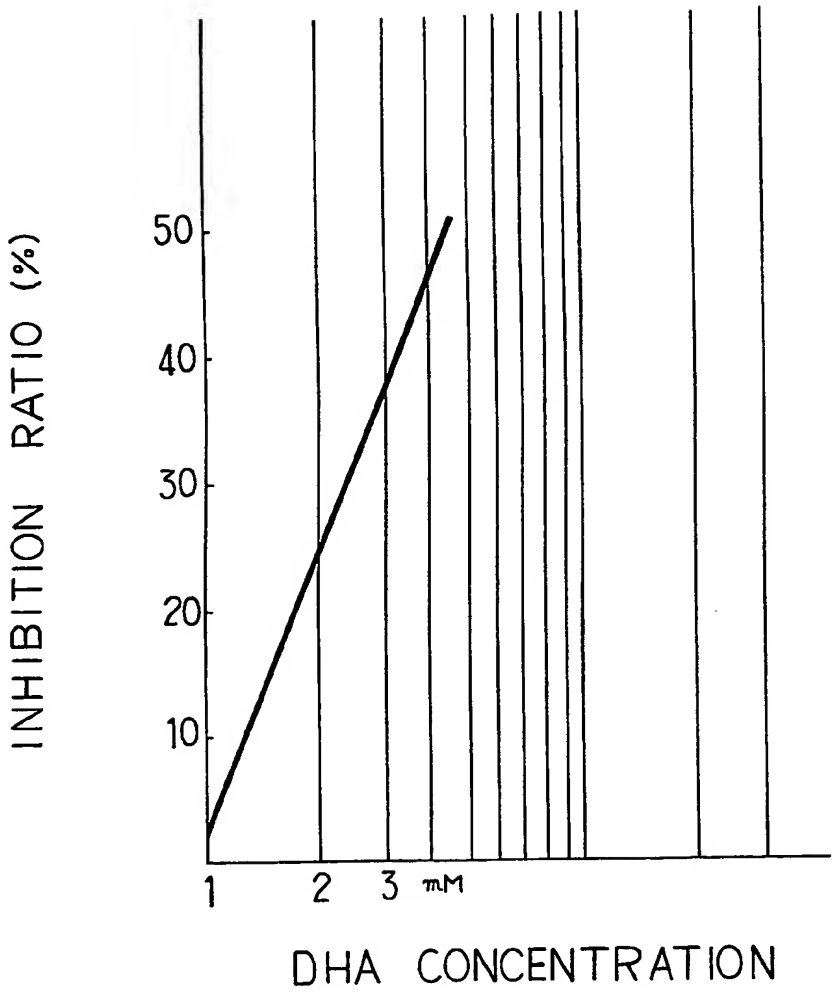
FIG.1



SPECIFICATION

Antithrombotic compositions containing docosahexaenoic acid

The present invention relates to a health food and a pharmaceutical preparation, and more particularly to a health food suitable for preventing circulatory diseases and to a preparation suitable especially for preventing or treating thrombosis among the circulatory diseases.

As the intake of land animal fats increases in dietary life, the incidence of various diseases of the circulatory systems, such as hyperpiesia, arteriosclerosis, cardiac infarction, stenocardia, adiemorrhysis, and the like, tends to increase. A change in vasculature is certainly one of the generative causes of such ischemia cardio diseases and adiemorrhysis or apoplexy, but formation of thrombus plays a particularly important role. In such diseases accompanying the formation of thrombus, it is important to reduce the blood agglutination ability by improving substantially the blood agglutination characteristics per se and thus an effective measure therefor has been direly sought.

All of the pharmaceutical agents which have hitherto been proposed and widely employed till now for treating diseases accompanying thrombus are of the nature of inhibitors of vascular contraction, and, as such, they are mere agents for symptomatic treatment. In other words, no radical measure to combat thrombotic diseases, including their prevention, has yet been found.

On the other hand, it has been known in the art that the intake of fish oils somewhat prolongs the life of patients of thrombo-cardiac diseases and that a large intake of fish and/or marine mammals shows a tendency to inhibit the generation of thrombo-cardiac diseases. For instance, A. M. Nelson et al, having administered fish oil to their patients and carried out a follow-up investigation over a long period, reported that the mortality rate of the patients given fish oil was noticeably lower by comparison with that of the control group ("Geriatrics" *33*, page 103, 1972). Dyerberg et al have also reported on the basis of their epidemiological research on Greenland Eskimos that the low incidence of thrombo-diseased patients among the Eskimos lies in the specific fatty acids composition of their blood, namely that their blood shows a low concentration of arachidonic acid and a high concentration of eicosapentaenoic acid, due to their large intake of fishes and marine mammals ("Lancet II" pages 15 to 17, 1978). Further, Siess et al had each white adult take mackerel in an amount of 500 to 800 g/day for 6 days and carried out tests on the agglutination ability of blood platelets and on the fatty acids composition in the blood plasma, by which they demonstrated that the agglutination ability decreases and the concentration of eicosapentaenoic acid in the blood plasma increases, namely that a negative interrelation exists between the ratio of eicosapentaenoic acid to arachidonic acid in the blood plasma (EPA/AA value) and the agglutination ability of blood platelets ("Lancet I" page 441, 1980).

In studying the special characteristics of fatty acids composition in aquatic animals and plants by taking the said known facts into consideration, it is noted that the oils and fats of aquatic animals and plants contain larger amounts of higher unsaturated fatty acids in comparison with those of land animals and plants and that the double bonds of these higher unsaturated fatty acids lie in ω-3 position.

Of the specific higher unsaturated fatty acids, the relation between eicosapentaenoic acid (EPA) and its preventive action on blood platelet agglutination is presently being studied as previously mentioned but no study has been made on docosahexaenoic acid [all cis - 4,7,10,13,16,19 - docosahexaenoic acid (22:6 ω-3)] (hereinafter referred to merely as "DHA") which is one of the higher unsaturated fatty acids similar to EPA.

Taking note of this fact, the present inventors give to each healthy adult 5 g/day of sardine oil over a one-month period and studied the relation between the fatty acids composition in blood plasma and blood platelets agglutination ability, by which they found that platelets agglutination due to adenosinediphosphate (ADP), collagen or the like is noticeably restrained by administering sardine oil and that a negative interrelation exists between the amount of DHA and blood platelets agglutination.

It has hitherto been known that linolic acid, linolenic acid and many other fatty acids show *in vitro* an anti-agglutinating action on blood platelets but it has been established that this does not mean that such fatty acids can restrain the formation of thrombus *in vivo*, since these fatty acids may change *in vivo* into arachidonic acid (AA) which may, in turn, change into tromboxane A_2 which is known to exert a most powerful agglutinating action on blood platelets.

Therefore, the present inventors administered a purified DHA and its derivative such as a glyceride or the like to rats to check the fatty acids composition of the blood and confirmed that the DHA concentration in blood plasma increases without showing any change of AA concentration therein and that the agglutination ability of the blood platelets and adhesiveness thereof decrease; and in other words, the administration of DHA effectively prevents blood agglutination *in vivo* without changing into AA.

Further, it was confirmed that DHA can be handled like conventional edible oils and fats and thus may well be utilized as a material for processing into various foodstuffs.

Therefore, an object of the present invention is to provide a highly beneficial health food that can be taken naturally in a form quite similar to ordinary food without requiring any special effort, unlike the conventional pharmaceutical preparations, patient's food or the like, whereby prevention or treatment of thrombosis and other blood disorders of the circulatory system can be effected.

Another object of the present invention is to provide a pharmaceutical preparation suitable for preventing or treating thrombosis.

According to the present invention, these objects can be attained by a health food comprising at least one of the docosahexaenoic acids or their deriva-

tives concentrated and isolated from the oil and fats extracted from aquatic animals and plants, or by a pharmaceutical preparation comprising at least one of the said compounds as an effective component thereof.

According to the present invention, DHA in the form of free acid or its derivative can be used singly or as a mixture. The derivatives may be a salt such as sodium, potassium or the like salt; an ester such as methyl ester, ethyl ester or the like; mono, di or triglyceride; or an amide.

The following may be listed as the raw material aquatic animals and plants for obtaining DHA and its derivatives: fishes such as mackerel, mackerel pike, sardine, anchovy, menhaden, codfish, shark and the like; crustacea such as crab, lobster and the like; aquatic mollusca such as cuttlefish, octopus, shellfish and the like; marine mammals such as seal, whale and the like; zooplanktons such as euphausia, copepoda and the like; phytoplanktons such as dinoflagellata and the like; and algae such as Chlorella and the like. The raw material selected must be of good freshness and quality. Extraction of DHA from such raw materials may be carried out by one of the conventional methods, such as a boiling extraction method, solvent extraction method, digestion method or the like. It is preferable to carry out the extraction within as short a time period as possible and under low temperature to avoid formation of peroxides and polymerization products and to store the extracted oil under low temperature and shutting out of air, to prevent a possible deterioration of quality. Subsequent concentration and isolation can be carried out by utilizing the physicochemical properties of DHA, namely through wintering, acidolysis or the like for glyceride type DHA, or wintering, urea addition, column chromatography, distillation or the like for ester type DHA. It is, of course, preferable to remove the accompanying arachidonic acid which converts into tromboxane A_2 and shows in vivo a strong blood agglutination action, but a suitable combination of said concentration and isolation operations enable obtaining DHA or its derivative which contains almost no arachidonic acid.

The resulting concentrate of DHA or its derivative is, if necessary, further subjected to an adsorption or distillation treatment known per se to remove unpleasant smell, colour, peroxides, polymerization products, non-saponified substances, waxes and other impurities.

It is important also for the said series of concentration, isolation and purifying treatments to be carried out under as low a temperature as possible and in an inert gas stream to avoid possible changes in quality due to oxidation, polymerization and other causes.

The resulting concentrate of DHA or its derivative has a high purity, a low oxide value of less than 5.0 milli equivalent/kg or less, a low non-saponified substance content of 0.3% or less and having a good taste and flavour as well is thus possessed of the desirable characteristics for use in foodstuffs.

The free acid type DHA was normally in the form of all cis - 4,7,10,13,16,19 - docosahexaenoic acid (C_{22}:6, ω-3) having a molecular weight of 328. The refractive index of methylester type DHA was n^{20}_D 1.49736.

DHA and its derivatives are apt to be oxidized and thus it is preferable to employ an antioxidant. As the antioxidant, tocopherol, dl - α - tocopherol, BHT, erythorbic acid or the like may be used. If DHA or its derivative is made into food or is added therein, a synergist such as citric acid, lecithin, ascorbic acid or the like may also be used.

When making the concentrate of DHA or its derivative into a foodstuff, the concentrate may be sealed in a gelatin or other capsule together with an above-described antioxidant and/or synergist to obtain an encapsulated foodstuff.

DHA and its derivatives can be handled in a manner similar to that for conventional or ordinary edible oils and fats and thus it can be added in various foodstuffs which contain any oil or fat with the use of a conventional method such as kneading, mixing, emulsifying, spraying, applying, injectional addition, dipping or the like, or employed as a part of the raw materials for preparing a cooking oil, seasoning oil, cooking sauce, dressing, mayonnaise, butter, margarine or the like. The amount of DHA concentrate required to be used or added can be determined by taking into consideration the advisable daily dosage per average adult which is 1.0 to 1.5 g/day. In the case of adding the concentrate to a foodstuff, if there is any fear that the taste or flavour of the selected foodstuff may be affected, a taste or seasoning, spice, perfume, smell modifier or the like can be added to solve the problem.

Any foodstuff to which DHA or is derivative has been added should preferably be sealed in a package that is impermeable to air and light, whereby the foodstuff can be stored over a relatively long period without causing quality deterioration.

It is preferable to administer the pharmaceutical preparation of the present invention through the oral route, but the preparation may also be administered by hypodermic, intermuscular or intravenous injection, or via the rectum as a suppository. For the purpose of oral administration, the concentrate may be sealed in a soft edible capsule to make a capsule preparation, or made in the form of a powder or granule preparation with the use of a suitable carrier such as kaolin, calcium phosphate, starch, crystallized cellulose or the like, or in the form of a tablet by further treating the powder or granule in a conventional manner. For the purpose of injectional administration, the concentrate is added and stirred in water together with an allowable amount of a surface active agent to form an oil-in-water emulsion.

The effective dose of DHA or its derivative on a concentrate basis is 1.0 to 1.5 g/day for adult persons, but the amount may be decreased for disease preventive use or continuous administration over a long period.

In the accompanying drawings:

Fig. 1 is a graph showing the relation between the restraining ratio and concentration of docosahexaenoic acid (DHA) relative to the blood platelets agglutination action of 1 μM of adenosinediphosphate (ADP); and

Fig. 2 is a graph showing the relation between the blood platelets agglutination restraining ratio and

concentration of docosahexaenoic acid (DHA) when 25 μg/ml of collagen is used as the agglutination agent in lieu of ADP.

The present invention will now be further explained with reference to Examples for preparing foodstuffs and Tests on pharmaceutical effects.

Examples of Food Preparation

Example 1

100 kg of mackerel fillets which were obtained by defrosting mackerels which had been frozen immediately after being caught were subjected to a boiling method to obtain 15 kg of mackerel oil. The oil was saponified for 2 hours by treating with a 10% NaOH-ethanol solution, and then the resulting mixture was acidified by adding hydrochloric acid to obtain 9 kg of fatty acid mixture. The mixture was then subjected to wintering at −60°C to obtain 4 kg of a higher unsaturated fatty acids concentrate. The concentrate was treated at 10^{-2} mmHg with the use of a flow-down type molecular still and then the remaining fraction was further concentrated by column adsorption chromatography to obtain 900 g of DHA concentrate. A gas chromatography showed a purity of 82%.

175 g of steam boiled flaked meat of tuna, 40 g of a seasoning liquid, 5 g of the DHA concentrate and 0.1 g of tocopherol were charged in a can, sealed under de-airing and sterilized by heating the can at 110°C for 70 minutes to obtain a canned tuna flake product. The product was subjected to a panel test together with a commercially available canned tuna flake product. The results obtained were as shown in the following table which revealed that no significant different in taste and flavour existed between the product of the present invention and the commercial product, even at 1 percent of the rate of risk.

Table 1

Number of testers: 30 persons

	taste	*smell*
Number of persons who liked the product of the present invention	19	14
Number of persons who like the commercial product	11	16

Example 2

1000 g of a cuttlefish oil extracted from the internal organs of cuttlefish by the autolysis method were hydrolized through a 3 stage treatment with the use of lipase and phospholipase to obtain 250 g of fatty acids mixture. The mixture was subjected to silica-gel chromatography to obtain 58 g of DHA fraction. This fraction showed a purity of 75%.

100 weight parts of minced pollack, 3 weight parts of table salt, 0.5 weight part of sugar, 1.2 weight parts of sodium glutamate, 3 parts of sweetened Japanese sake (rice wine), 3 parts of egg white fresh, 3 parts of the said DHA fraction or concentrate and 0.3 part of Vitamin E preparation were kneaded with the use of a kneading machine, moulded on a wood plate, and then boiled with steam at 90°C for 50 minutes to obtain a boiled fish paste product "kamaboko". A panel test showed that the resulting boiled fish paste possessed a good flavour and mouth feel which could not be distinguished from a comparable commercial product.

Example 3

Oil obtained from the edible parts of fresh sardines through a boiling method was saponified by a 10% NaOH-ethanol solution and then acidified with use of hydrochloric acid to obtain a crude DHA in free acid type. The crude DHA was purified with the use of silver nitrate-containing silica-gel column chromatography to obtain a DHA concentrate having a purity of 95%. 10 weight parts of the resulting DHA concentrate was mixed with 1 part of the said sardine oil and the mixture was subjected to acidolysis and then the resulting triglyceride was separated. A gas chromatographic analysis showed that the DHA in the fatty acids composition of the triglyceride was 85%.

The triglyceride with the high content of DHA was fed into a double cylinder type capsule former to obtain a capsuled food each capsule containing 0.4 g of the said triglyceride hermetically reeled in a gelatin-glycerine membrane.

The capsuled food is easily swallowed with water without any noticeable of oily taste or smell.

Example 4

1000 kg of sardines which had been ice-chilled immediately after having been caught was headed and gutted within 5 hours after being caught and subjected to a boiling method to obtain 100 kg of sardine oil. The oil was saponified by treating for 2 hours with a 10% NaOH-ethanol solution and acidified with hydrochloric acid to obtain 60 kg of fatty acids mixture. The mixture was distilled under vacuum of less than 1 mmHg to obtain 10 kg of a remaining fraction. The fraction was then subjected to the acidolysis reaction with 1 kg of sardine oil to obtain a triglyceride containing DHA in a high amount. A gas chromatography showed that DHA comprised 55% in the fatty acids composition of the triglyceride.

60 weight parts of milk, 20 weight parts of sugar, 17.5 weight parts of egg yolk, 0.8 weight part of vanilla essence, 0.1 weight part of tocopherol preparation, 0.1 weight part of glycerine fatty acid ester and 1.5 weight parts of the said triglyceride containing DHA in high amount were mixed under heating. The resulting hot mixture was filled in pudding cups of synthetic resin material, in each of which a suitable amount of caramel syrup had been charged in advance. The mixture in the cups was boiled by steam at 80°C for 15 minutes to obtain a custard pudding product. The product was subjected to a panel test which confirmed that the product had a good taste, flavour and mouth feel indistinguishable from a comparable commercial product.

Example 5

To 10 kg of a sardine oil obtained by boiling the edible parts of sardines, 100 g of sodium methylate and 3 litres of ethanol were added to cause an esterification at 20°C for 6 hours under stirring. After having completed the reaction, the mixture was left to stand to remove a precipitate of glycerine, neutralized by 1/10 N–H_2SO_4, sufficiently washed with water and then subjected to a centrifugal separation under 10000 x G to obtain 9.5 kg of a mixture of fatty methylates. The mixture was distilled under vacuum

of 1 mmHg or less to distill out an initial fraction to obtain 950 g of a remaining fraction. The remaining 950 g fraction was dissolved in 4.75 kg of n-hexane and treated with an activated silicic acid column to remove impurities of peroxides, polymerization products and the like and then distilled off the n-hexane to obtain 930 g of a methyl ester containing DHA in a high amount. A gas chromatography showed a 72% DHA purity.

28.2 weight parts of minced pork, 41.5 weight parts of onion, 5.4 weight parts of starch, 10.0 weight parts of wheat flour, 7.5 weight parts of shiitake (a species of Japanese mushroom), 1.0 weight part of table salt, 1.0 weight part of a Japanese soy sauce, 0.3 weight part of pepper, 0.2 weight part of ginger, 0.9 weight part of sugar, 0.3 weight part of a chemical seasoning, 2.0 weight parts of sesame oil and 4.7 weight parts of the said methyl ester with high DHA content were mixed in a mixer. The resulting mixture was fed into a moulding machine to form Chinese shaomais (a kind of dumpling), each weighting 15 g. The moulded shaomais were boiled in a continuous boiling machine at 96°C for 13 minutes and then cooled by blown air. The resulting food product was quick-frozen at −30°C with the use of a spiral conveyer type continuous freezing machine and then hermetically packaged with a polyamide resin laminated film to obtain a frozen shaomai product.

After having been stored for period of 3 months at −25°C, the frozen shaomai was boiled with steam at 96°C for 15 minutes and then subjected to a panel test together with a comparable commercial shaomai product which confirmed that the product of the present invention possessed a good taste and flavour quite similar to the commercial product.

Example 6

10 kg of frozen Alaskan red salmon heads was defrosted, treated with a chopper, freeze-dried to reduce the water content thereof to 5% or less, and then was extracted the head oil with the use of 8 kg of n-hexane. The hexane solution containing the head oil was left standing over night at −45°C. After filtering off the solid fat formation, the filtrate was treated with an activated silicic acid column to remove peroxides, polymerization products and other impurities, and then the n-hexane was distilled out to obtain 0.7 kg of an essential oil containing DHA triglyceride as its main component. A gas chromatography showed the DHA content of the essential oil to be 29.5%.

0.7 kg of the said essential oil and 1.4 g of α-tocopherol were mixed and the resulting mixture was fed into a capsule former wherein the mixture was sandwiched between 2 gelatin-glycerine sheets which were then the sheets were pressed and concurrently stamped out by a die from both outer sides to form a capsuled food product, each containing 0.4 g of the said essential oil.

The resulting capsuled food was subjected to a panel test which confirmed that the capsule can easily be swallowed with water without any noticeable oily taste or smell of fish.

Example 7

1 kg of commercially available vegetable juice, 1.5 g of carrageen, 1.5 g of fatty acid sugar ester, 1.5 g of monoglyceride (emulsifier), 4.5 g of sugar, 10 g of squeezed lemon, 0.8 g of lemon essence and 50 g of an essential oil of DHA glyceride concentrate obtained by a method similar to that as described in Example 6 were homogenized by mixing for 5 minutes with the use of a juicing mixer. The resulting vegetable juice was chilled at 5°C and then subjected to a panel test which found that the juice had no oily taste or smell and possessed a good taste and flavour indistinguishable from commercially sold vegetable juice products.

Example 8

10 kg of the oil and fat extracted with *n*-hexane from freeze-dried antarctic euphausia was hydrolyzed with the use of lipase and phospholipase to obtain 6.1 kg of a fatty acids mixture. The mixture was subjected to molecular distillation under 10^{-3} mmHg with the use of a flow-down type molecular distillator and then the remaining fraction was concentrated and isolated with the use of a silica gel chromatography to obtain 1 kg of DHA concentrate. A gas chromatography showed the concentrate had a DHA purity of 82%.

8.5 g of the said DHA concentrate, 47 g of potato, 10 g of green pease, 10 g of carrot, 1.5 g of seasoning and 0.1 g of tocopherol were charged in a No. 4 size can, sealed under de-airing and heat-sterilized at 110°C for 50 minutes to obtain a canned salad product. The product was subjected to a panel test which confirmed that the product had a good taste indistinguishable from that of comparable commercial products.

Test Examples of Pharmaceutical Effects

Animal Tests

The pharmaceutical effects of the agents used in the present invention will now be shown with animal tests. In each of the tests, DHA of 98% purity was used, which was obtained by boiling the edible parts of sardines, ethyl esterifying the resulting oil with the use of sodium ethylate, and then purifying the resulting ethylate with the use of a silver nitrate-containing silica gel column chromatography.

Test 1

No food was given to healthy rabbits (white species in Japan) for 24 hours to obtain blood from their circular veins. To the blood, 1/10 volume of 3.8% sodium citric acid solution was added and then the resulting mixture was subjected to a centrifugal treatment at 1000 rpm for 10 minutes to obtain an upper layer as a blood plasma rich in blood platelets (PRP), the remaining lower layer being further subjected to another centrifugal treatment at 3000 rpm for 10 minutes to obtain an upper layer as a blood plasma poor in blood platelets (PPP). Using a conventional blood platelet agglutinator and according to the Born and O'Brien method, agglutination causing substances (ADP and collagen) of a constant concentration were added, kept at 37°C, stirred and then recorded the increase of transparency resulting from the agglutination was recorded on by a recorder incorporated in the said agglutinator. DHA was added 6 minutes before the addition of the said agglutination-causing substance.

The results were shown in Figs. 1 and 2. From the graphs of Figs. 1 and 2, the following points are evident:

On ADP agglutination (1 μM) and collagen agglutination (2.5 μg/ml), DHA shows a restraining action at 1.2 and 3mM respectively, which action depends on the amount added and is equivalent in extent to that attainable by EPA.

The DHA causes with 2 mM a parallel movement of the ADP agglutination curve (0.5, 1.0, 2.0, 4.0 and 8.0 βM) and the collagen agglutination curve (1.25, 2.5, 5.0, 10.0 and 20.0 μg/ml) to the higher concentration sides to show a conflicting antagonism which is equivalent to that of EPA.

On ADP agglutination (1 μM) and collagen agglutination (2.5 μg/ml), the effect of the combined action of 1.5 mM of DHA and 1.5 mM of EPA is equivalent to the restraining action attained singly with 3mM of DHA or EPA.

Test 2

Healthy wistar male rats of 6 weeks were classified into the following 4 groups, each consisting of 10 rats.

Table 2

Group 1	ordinary food	water 2 ml/kg
Group 2	food rich in cholesterol	ditto
Group 3	ditto	DHA 100 mg/kg
Group 4	ditto	EPA 100 mg/kg

To rats in groups 3 and 4, the agents of DHA or EPA were administered orally continuously and compulsorily over a period of 30 days in the amount of 100 mg/kg per day.

After 30 days of administration of the said agents, no food was given to the rats and on the next day, they were ether-anesthetized to obtain blood from the aorta of the lower abdominal region. PRP and PPP samples were obtained from the blood similar to the manner in vitro as described in Test 1. The blood platelets agglutination ability was measured by adding blood platelets agglutination-causing agents (ADP and collagen) of a constant concentration to PRP, and, further, the total cholesterol concentration (T-Ch) in the blood serum was measured.

The results were shown as in the following Table 3.

Table 3

Group	*ratio of blood platelet agglutination (%)* ADP (2 μM)	collagen (4 μg/ml)	*T-Ch concentration in serum (mg/dl)*
1	38.4 ± 4.7	48.6 ± 5.3	61.45 ± 5.63
1	38.4 ± 4.7	48.6 ± 5.3	61.45 ± 5.63
2	51.6 ± 6.2	59.8 ± 5.8	206.41 ± 31.12
3	39.3 ± 3.6	43.7 ± 4.5	124.35 ± 32.48
4	40.7 ± 5.1	42.3 ± 4.4	111.25 ± 25.55

From the above results, the following conclusions can be drawn:

On ADP agglutination (1-10 μM) and collagen agglutination (1-10 μg/ml), the administration of DHA 100 mg/kg (Group 3) clearly restrains apparently the blood platelet agglutination in comparison with the high cholesterol food group that was not given DHA Group 2, and the effect thereof is equivalent to that of the group which was administered EPA 100 mg/kg (Group 4). In the high cholesterol food group of Group 2, an increase in blood platelet agglutination was recognized in comparison with the ordinary food group of Group 1.

The T-Ch concentration in the high cholesterol food groups of Groups 2 to 4 is higher than that in the ordinary food group of Group 1.

The above fact shows that DHA has an anti-agglutination action on blood platelets and that its effect is equivalent to that of EPA. In the tests, the following foods were employed.

Ordinary food:

A solid animal food manufactured under the trademark of "MF" by the Oriental Yeast Industry Co., Ltd. of Japan.

Food rich in cholesterol:

Prepared by mixing the following ingredients.

Table 4

casein	18 (%)
sucrose	61
cellulose	4
hardened plant oil	10
vitamines	2
minerals	4
cholesterol	0.5
sodium cholate	0.5

Test 3

Hartley male guinea pigs of 800 to 1000 g weights were classified into 2 groups, each consisting of 10 guinea pigs and fed on ordinary solid food sold under the commercial product name of "RC-4" by the Oriental Yeast Industry Co., Ltd. of Japan. The control group and the agent-administered groups were given a single daily dosage of physiological salt solution and 200 mg/kg of 98% DHA, respectively, for a period of 2 weeks. The dosages of DHA were prepared by emulsifying DHA in purified water with the use of a Potter's homogenizer and compulsorily administered with the use of an oral probe.

The test guinea pigs were placed in a temperature controlled room at 50°C for 5 minutes, and their auricular veins were pieced with an injection needle of 1 mm diameter to cause bleeding. The bleeding blood was absorbed on a filter paper at intervals of 5 seconds to measure the time period required until the bleeding stopped. In this case, when a colorless solution is absorbed by the filter paper. The bleeding

time was determined as being the time which trancepired until the red colour of the blood no longer showed on the filter paper.

The bleeding time was measured for the veins of both ears and the mean value was taken as the true bleeding time.

After 2 weeks from the first administration, the bleeding time of each group was measured, by which it was found that a noticeable prolongation of the bleeding time was registered by the DHA-administered group as compared with that of the group which was given a physiological salt solution, as shown below:

Table 5

Group administered physiological salt solution	109.5 ± 7.8 seconds
Group administered DHA	132.6 ± 11.2 seconds

Clinical Tests

Test A

Contents of the canned vegetable salad obtained by the process as described in Example 8 were given to each of 8 healthy male adults in an amount of 1 canned product/day for a period of 7 days and the results of the test were as shown in the following Table 6.

Table 6

Identification No. of the tested person	*Before and after taking canned product*	*Docosahexaenoic acid (μg/ml of blood plasma)*	*Docosahexaenoic acid/-Arachidonic acid*	*Blood platelets agglutination ability (ADP agglutination threshold value, μM)*	*Adhesive coefficient of blood platelets (%)*
1	before taking	108.4	0.55	1.0	30.3
	after 7 days	212.3	1.02	2.0	0.5
2	before taking	58.9	0.41	1.0	80.9
	after 7 days	113.7	0.86	3.0	14.2
3	before taking	48.7	0.36	2.0	72.6
	after 7 days	268.4	1.43	2.0	13.4
4	before taking	32.4	0.37	2.0	52.5
	after 7 days	135.5	1.11	2.5	22.1
5	before taking	42.1	0.34	1.0	69.3
	after 7 days	114.3	0.83	1.5	45.9
6	before taking	74.6	0.69	1.0	49.8
	after 7 days	161.9	1.24	3.0	47.5
7	before taking	54.6	0.45	3.5	58.5
	after 7 days	259.8	1.81	4.0	49.5
8	before taking	37.1	0.37	3.5	41.8
	after 7 days	91.8	0.83	3.5	28.3
mean value	before taking	57.1	0.44	1.9	57.0
	after 7 days	170.3	1.17	2.7	27.7

As apparent from the Table 6, the DHA concentration and the ratio thereof to arachidonic acid increase by about 3 and 2.7 times in mean value respectively, after taking the canned salad product and remarkable improvements were observed in the agglutination ability and adhesiveness of blood platelets, both of which have a close bearing on thrombosis and other circulatory diseases.

Test B

The capsule food as described in Example 3 was given to each 10 healthy male adults in a dosage of 3 capsules/day over a period of 4 weeks, the results of which test were as shown in the following Table 7:

Table 7

Take-in of capsule food	*mean values of 10 adults tested* before taking	after taking
DHA (μg/ml of blood plasma)	60.3	150.8
DHA/AA	0.41	0.94
Blood platelets agglutination Ability (ADP agglutination threshold value, μM)	1.5	1.8
Blood platelet adhesion coefficient (%)	54.4	30.2

As is apparent from the foregoing Table 7, the DHA concentration and the ratio thereof to arachidonic acid increased by about 2.5 and 2.3 times in mean value respectively, after taking the capsuled food and remarkable improvements were observed in the agglutination ability and adhesiveness of blood platelets, both of which have a close bearing on thrombosis and other diseases of the circulatory system.

CLAIMS

1. A pharmaceutical composition or foodstuff which contains docosahexaenoic acid or a derivative thereof.
2. A composition or foodstuff as claimed in Claim 1, wherein the derivative is a salt, ester or amide of the acid.
3. A composition or foodstuff as claimed in Claim 1 or 2, which also contains an antioxidant.
4. A composition or foodstuff as claimed in Claim 1, 2 or 3, wherein the docosahexaenoic acid or derivative was obtained from an aquatic animal or plant.
5. A composition as claimed in Claim 4, wherein the acid or derivative was obtained by extraction, concentration and isolation from the animal or plant.
6. A pharmaceutical composition as claimed in any preceding claim, in the form of a capsule, powder, granules, tablet or injectable emulsion.
7. A foodstuff as claimed in Claim 1, substantially as hereinbefore described in any of Examples 1 to 8.

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